

Article

Analysis of Dairy Cow Behavior during Milking Associated with Lameness

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Abstract: The detection of lame cows is a challenging and time-consuming issue for dairy farmers. Many farmers use the milking time to monitor the condition of their animals. Because lame cows often show increased stepping when standing to relieve pressure on aching claws, we investigated whether lame cows showed increased activity in the milking parlor. On 20 Swiss dairy farms, 647 cows were scored on lameness with a five-point locomotion score and categorized as clinical lame and non-lame cows in order to see if there are differences in behavior between these two groups (non-lame = scores 1 and 2; lame = scores 3, 4, and 5). During one evening milking, the behavior of the cows was analyzed. A three-dimensional accelerometer, attached to the milking cluster, detected the hind leg activity indirectly via the movements of the milking unit. Additionally, head movements, as well as weight shifting and the number of steps with the front legs, were analyzed from video recordings. Owing to a high percentage of false positive hind leg activities in some milkings measured by the sensor, only 60% of the collected data were evaluated for behavior (356 cows/milkings on 17 farms). Twenty-seven percent of the investigated cows were classified as lame. The lameness prevalence was increasing with increasing parity. Lame cows showed a higher hind leg activity during milking as well as a higher frequency of front steps and weight shifting events during their stay in the milking parlor than non-lame cows. No relation between the status of lameness and the number of head movements could be seen. Observation of increased stepping and weight shifting of individual animals during milking by the farmer could be used as an additional indicator to detect lame cows, but further investigations are required.

Keywords: milking parlor; parity; accelerometer; stepping; head movement; lameness prevalence



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1. Introduction

Lameness represents an increasing problem in dairy herds kept in free-stall housing [1,2]. According to Becker et al. [1], in 2010 the prevalence of lameness in Swiss dairy cows reached about 14.8% on cow level and 80.8% on farm level with a locomotion score ≥ 2 , defined as clinical lameness. In Canadian dairy free-stalls, the lameness prevalence (locomotion score ≥ 3) amounted to about 28.3% on cow level in 2019 [3]. Even moderate lameness leads to economic losses caused by declining milk yield [4,5], decreased fertility [6,7] and increased susceptibility to diseases in general [8]. Besides the economic aspects, lameness also has a negative impact on animal welfare owing to pain and movement restrictions [9], which is reflected in changes in the daily behavior of the affected animal, such as reduced locomotion activity, shorter eating time, or longer lying periods [10].

The standard method for lameness assessment still consists of visual observation and assessing the individual animal while it is walking, for example with the five-point locomotion score developed by Sprecher et al. [6]. Another technique, often applied in the context of functional claw trimming, is the observation of the foot positions from behind when the cows are fixed in the feeding fence [11,12], as introduced by Raven [13] to

detect misalignment and uneven claw weight-bearing. The attempt to relieve aching claws may lead to weight shifting and increased stepping behavior during times of standing idle [14–17]. However, owing to their stoic nature, cows with moderate lameness are often difficult to detect visually, even under optimal observation conditions. Depending on the herd size and on which and how many claws are affected, the diagnosis of lame cows through direct observation is time consuming and requires expertise from the observer. Therefore, to improve lameness detection in modern dairy housing systems, many different technical approaches have already been tested, most of them with the aim to detect lameness automatically [18,19].

Considering that lame cows try to relieve their painful limbs by distributing and shifting their weight, Rajkondawar et al. [20], Pastell et al. [21], and Chapinal and Tucker [14] tried to measure the weight distribution of cows while walking and standing with the aid of special pressure plates on the ground. Video analysis of two- and three-dimensional videos with computer vision and machine learning to detect irregularities in the cow's gait or back posture has been applied by many different scientific groups [22–25]. Attaching accelerometers to different parts of the cow's body allows the measurement of leg movements during a gait cycle or providing a closer inspection of her lying behaviors [26,27]. Both the moving and the resting behavior may differ between lame and non-lame cows and therefore provide support to detect lame individuals within a herd. To gain a wider view on behavioral changes in lame cows kept in free-stalls, Weigele et al. [10] applied a combination of video observation and analysis of accelerometer data. In a subsequent step, Riaboff et al. [28] even used a combination of accelerometer records and global positioning system data to identify discriminating behavioral and movement variables in lame cows on pasture.

Because lame cows show increased stepping and weight shifting during prolonged standing compared with non-lame cows [14–17], this knowledge could be used to detect lameness during milking in the parlor, as attempted in a recently published study that used cow-related indicators for detecting lame cows in the parlor [17]. Cows milked in a parlor have prolonged standing times in the waiting area and can be observed individually during the milking process. In addition to the studies of Werema et al. [17] and Pastell et al. [21], there have been only a few attempts to analyze the behavior of lame cows in the milking parlor so far, although it is common practice for farmers to assess lameness through direct observation of cows during milking [29].

Therefore, the objective of this study was to compare the behavior of lame and non-lame cows in milking parlors in order to determine whether lameness could be related to behavioral changes in general and, in particular, to an increase in leg movements during the milking process.

2. Materials and Methods

During two winter feeding periods, a study on detection of subclinical mastitis was conducted on 20 Swiss dairy farms. Within this study, lameness was an exclusion factor for participating cows. Upon review, the relationships between clinically lame and non-lame cows described in the present paper were found and are now published separately.

2.1. Farm Recruitment and Selected Milkings

The data collection took place from November 2021 to March 2022 and from October to December 2022. Twenty dairy farms, distributed throughout Switzerland, participated in the study. In order to recruit the study farms, for the first period, an information letter about the project was sent by the three Swiss cattle breeding associations to their members (Braunvieh Schweiz, Zug; swissherdbook, Zollikofen; and Holstein Switzerland, Posieux). For the second period, an article about the ongoing project was published in Swiss specialized magazines for agriculture and cattle breeding (Schweizer Bauer, Bern; Braunvieh Schweiz, Zug; and swissherdbook bulletin, Zollikofen). To participate in the framework study, the responding farms had to meet some requirements. The selection

criteria were that cows were kept in free-stalls, the farm had issues with the occurrence of subclinical mastitis on herd level, the tank milk count was more than 100,000 somatic cells per milliliter for at least three months, and the cows were milked in an auto-tandem parlor with a permanently mounted milk meter twice a day. Furthermore, every cow had to have an individual collar number for quick identification, and the structural conditions of the barn had to be suitable to assess lameness after the cows left the milking parlor. In total, data from 647 cows were collected. The dairy breeds were Holstein or Holstein \times Swiss Fleckvieh (70.3%), referred to as Holstein in the following, Brown Swiss (27.5%), and a variety of others (2.2%). The herd sizes varied between 19 and 60 lactating cows. Seven of the milking parlors were from the company GEA (GEA Group, Düsseldorf, Germany), twelve from DeLaval (DeLaval, Tumba, Sweden), and one from Milkline (Milkline, Podenzano, Italy). All milking machines except one worked with automatic cluster removal. The pulsation cycle was alternating for all of them.

Data from cows that suffered from acute mastitis, as well as data from newly purchased cows during their first week on the farm, were excluded because these criteria could directly influence the cow's behavior. Because a three-dimensional (3D) accelerometer recorded the motion of the milking unit to indirectly detect the hind leg activity (see Section 2.2.2 "Hind leg activity" below), measurements with affected movements of the milking cluster were also excluded from the further analyses. This occurred when the milker touched or held the milking cluster during milking as well as if the milking unit was attached a second time to the udder because the cow kicked off the milking unit or if by manual control of the udder filling level the proportion of residual milk in the udder was too high. In addition, if the flow-adjusted automatic stimulation started several times in between, these milkings could not be considered. The flow-adjusted stimulation is a form of automatic stimulation that adjusts the pulsation depending on the milk flow and thus causes a massage of the teats when the milk flow falls below a defined level. In addition, due to technical reasons regarding the data collection with the acceleration sensor, which will be explained in more detail in Section 2.2.2 ("Hind leg activity"), further milkings were excluded from the evaluation. Taking all these exclusion criteria into account, data from 356 cows from milkings on 17 farms were analyzed in the further examination.

2.2. Data collection and Parameters

2.2.1. Lameness Assessment

The lameness assessment was performed by the same trained researcher (D.S.), who observed the cows on their way through the walkways to the feeding fence after leaving the milking parlor. An adapted five-point scoring system, according to Sprecher et al. [6] and Thomsen et al. [30], as described in Table 1, was applied, from which clinical lameness reaches from locomotion score 3 (mild lameness) to 5 (severe lameness). In the applied system, a score of 2 is defined as "uneven gait without evident signs of lameness", which includes cows that do not fit into score 1, as they do not show a perfect gait, but are also not lame. It was of interest if a difference in behavior between clinical lame and non-lame cows could be seen. Therefore, referring to the binary classification introduced by Winckler and Willen [31], animals with lameness scores 1 and 2 were summed up in the category "non-lame", and those with a score from 3 to 5 were grouped in the category "lame".

Table 1. Applied adapted five-point locomotion scoring for dairy cattle according to Sprecher et al. [6] and Thomsen et al. [30] with binary categorization after Winckler and Willen [31].

| Category | Score and Brief Term | Description |
|----------|----------------------|---|
| Non-lame | 1. Normal | Normal gait pattern with no signs of irregularities or uneven weight bearing between legs. In most cases flat back while walking and standing. |
| | 2. Uneven gait | Cow walks almost normally but with a slightly uneven gait. No evident signs of lameness. Back may be arched while walking but flat when standing. |

Table 1. Cont.

| Category | Score and Brief Term | Description |
|----------|----------------------|--|
| Lame | 3. Mild lameness | Deviation from the regular gait rhythm owing to uneven load on the legs and shortened stride on one or more legs. In most cases arched back while walking and head carried lower than normal. Often difficult to be certain which leg is affected. |
| | 4. Lameness | Obvious lameness with in most cases arched back while walking and standing with clear attempt to unload one or more legs. In most cases head bob while walking. Usually obvious which legs are affected. |
| | 5. Severe lameness | Cow is reluctant or unable to bear weight on the affected leg while walking or standing. In most cases arched back while walking or standing and head bob while walking. |

2.2.2. Hind Leg Activity

The hind leg activity was measured indirectly by the movement of the milking cluster, based on the method developed by Raoult et al. [32]. Movements of the hind legs, such as foot-lifting, stepping, or kicking, are transmitted as oscillation to the milking cluster and thus can be recorded with the aid of a 3D-acceleration sensor. Therefore, a 3D-accelerometer (MSR 145 data logger, 20 × 15 × 52 mm, ~16 g; MSR Electronics GmbH, Seuzach, Switzerland) was attached to the top of the collection piece of each milking cluster. During one evening milking, the sensors recorded the movements of the milking clusters continuously.

Technical Details

The sensor detects acceleration of the milking unit in three dimensions (x = latero-lateral, y = cranio-caudal, z = dorso-ventral) with a frequency of 10 Hz, an accuracy of ± 0.15 g, a resolution of ± 0.03 g, and a range of ± 15 g ($1 \text{ g} = 9.81 \text{ m}\cdot\text{s}^{-2}$). The acceleration values measured by the 3D-accelerometer were read out by the MSR V6.06.02 software (MSR Electronics GmbH), which generates three curves consisting of the recorded deflections of the sensor in each of these three dimensions. Within this software each milking was determined manually, assigned to the individual cow and milking place number, and saved as a CSV-file. The individual milkings start with the attachment of the last milking cup to the cow's teats and end with the detachment of the unit. Both operations generate high acceleration values and are clearly visible on the curve progressions.

Subsequently, those single milkings were transferred to another evaluation software (Milking-Time-Test-Auswertung V1.0, InnoClever GmbH, Liestal, Switzerland), which was specially developed for the purpose of the detection of hind leg activity based on the evaluation method of Raoult et al. [32]. Lateral accelerations along the horizontal x -axis exceeding a manually set acceleration threshold of 0.25 g are detected as acceleration due to hind leg movements of the milked cow.

The threshold setting had been investigated in a pre-test where the sensor data of 36 milked cows had been compared with the results of direct observation of the cows' movements during milking in one BouMatic (BouMatic, Madison, WI, USA), two GEA (GEA Group, Düsseldorf, Germany), and two DeLaval (DeLaval, Tumba, Sweden) auto-tandem milking parlors. Within the pre-test, six acceleration thresholds, ranging from 0.15 to 0.40 g in steps of 0.05 g, were tested. In consideration of the sensitivity (0.70), the specificity (0.97), and the positive predictive value (0.59), a threshold set at 0.25 g showed the best results to detect hind leg movements of the cow (Appendix A). Based on the detected accelerations, the software creates so-called activity clusters. Accelerations that exceed the threshold and occur in an interval of less than three seconds are counted as one activity cluster. Thus, repetitive hind leg movements such as stepping are counted as one activity phase. These activity clusters can then be calculated as "number of activity phases

per minute”, hereafter referred to as hind leg activity (HLA), for each milking by dividing the number of clusters by the duration of the individual milking. In this way, HLA can be compared between individual cows regardless of milking duration.

Limitations of the Sensor-Measurement

A threshold set at 0.25 g will detect even small shifting and stepping events, which are often caused by the cow trying to shift her weight. However, some milking units show a high intrinsic motion, as mentioned in Section 2.1 (“Farm recruitment and selected milkings”). These intrinsic motions often occur in the context of high milk flows in combination with settings of the position arm of the milking unit. Owing to these intrinsic motions, a threshold at 0.25 g cannot be used for every milking machine in general because the amount of false positive detected HLA would be too high. However, a threshold higher than 0.25 g will not accurately detect small movements of the cow. Therefore, in order to detect movements such as weight shifting and stepping, which could indicate painful claws, each milking was manually checked for high intrinsic motion within the Milking-Time-Test software (Milking-Time-Test-Auswertung V1.0, InnoClever GmbH, Liestal, Switzerland). Here, 257 of 647 milkings (39.7%) presented high intrinsic motion and were excluded from further analysis.

2.2.3. Front Leg Activity and Head Movements

To assess the cow’s activity during milking, videos of each cow’s head and front legs were recorded simultaneously with HLA measurements during one evening milking. Therefore, cameras (Hero 7, GoPro, Inc., San Mateo, CA, USA) were installed inside the milking parlor with a side view on each milking stall in order to capture the cow’s head and front legs from the side. The halters for the cameras were installed two milkings beforehand to accustom the animals. For the framework study on milking technique and udder health, the cameras had to be focused on the front part of the animal with the aim to investigate the facial expression of the cow. Owing to some light conditions and designs of older milking parlors, this aim had to be discarded. The recorded videos were continuously evaluated using the free animal observation software “BORIS” [33], focusing on the front leg and head movements of the cows. The movements were divided into the behaviors as listed and described in Table 2. The video observation started when the cow had completely entered the milking stall and ended when the door opened in front of her and she left the stall. In contrast to HLA, which was measured by the acceleration sensor during the time the milking cluster was attached to the udder, the direct observation allowed us to assess the behavior during the entire stay of the cow in the milking stall. Behaviors resulting from aching claws are not restricted to the time of the cluster attachment. To obtain comparable parameters regardless of the milking duration, each activity was calculated as number of activities per minute by dividing the amount of each activity by the total observation time. This resulted in the following parameters for the statistical analysis: front steps (FS) per minute, horizontal head movements (HHM) per minute, and vertical head movements (VHM) per minute. Because some behaviors (rapid expulsive head movements, head twitching, head shaking, head scratching, and kicking with the front legs) occurred very rarely, no statistical analysis on these behaviors has been performed.

Table 2. Ethogram of behaviors assessed during video observation.

| Body Part | Behavior | Definition |
|------------|---------------------|---|
| Head | Horizontal movement | Head movement either to the left or the right side or back to the center, no matter how far the movement reaches |
| | Vertical movement | Head movement up or down into another range ¹ , no matter how many ranges the head crosses. Therefor dividing the cow's head position into four ranges ¹ : 1. Head and neck are held above the horizontal line of the back 2. Head and neck are equal or nearly equal to the imaginary straight line of the back 3. Head and neck are bent down at chest level 4. Head and neck are bent down nearly completely |
| | Hoicking | Big rapid expulsive head movement upwards |
| | Twitching | Tiny choppy head movement without changing the head position |
| | Shaking | Fast rhythmic circular head movements |
| | Scratching | Rubbing the head against a component of the milking box |
| Front legs | Stepping | Lifting or shifting the claw |
| | Kicking | Rapid expulsive movement of the claw with the aim of striking towards the abdomen or the milking cluster |

¹ After de Oliveira and Keeling [34].

2.3. Statistical Analyses

Statistical analyses were performed in RStudio Version R4.1.3 [35]. The lameness prevalence was determined descriptively. To investigate if parity has an influence on the proportion of lameness within a herd, we used a generalized mixed model (glmer(); "lme4" package; Bates, Mächler [36]) including lameness (factor with two levels: lame and non-lame) as outcome variable, parity (factor with five levels: 1 to 5+) as fixed effect, and farm as random effect.

$$\text{Lameness} \sim \text{Parity} + (1 \mid \text{Farm})$$

The *p*-value was evaluated using the anova() function from the "stats" package R-Core-Team [37], making a comparison between the model including parity as fixed effect and the model without any fixed effect. The 95% confidence intervals (CI95) of the proportion of lame cows per parity were calculated using the effect() function from the "effects" package [38,39].

To analyze the different behaviors, four linear mixed effects models (lmer(); "lme4" package; Bates, Mächler [36]) were used; one for each observed behavior (HLA, FS, HHM, VHM) as the outcome variable. Model assumptions (normal distribution) were tested by graphical residual analysis (simulateResiduals()); "DHARMA" package; Hartig [40]). All behavioral outcome variables were transformed by drawing the square root (sqrt). Lameness (factor with two levels: lame and non-lame) and parity (factor with five levels: 1 to 5+) were set as fixed effects. Because all interactions and days in milk as a fixed effect did not have a significant effect on the models (*p* > 0.05), they were excluded within stepwise model reduction. The random effect contained the individual milking stall (MS) nested in the farm. The models followed this formula:

$$\text{sqrt(Observed Behavior)} \sim \text{Lameness} + \text{Parity} + (1 \mid \text{Farm/MS})$$

The *p*-values were evaluated by comparing the impact of the fixed effect on the models using the anova() function from the "stats" package R-Core-Team [37]. The estimated means and the standard errors were calculated with the emmeans() function from the "emmeans" package [41].

3. Results

3.1. Prevalence of Lameness

Lameness was present on 19 of 20 farms (95%) in at least one of the lactating cows in the herd. In total, 174 of 647 scored cows (27%) were assessed as “lame”. The prevalence of lame cows per farm is shown in Figure 1. Of the 174 lame cows, 128 cows were scored with lameness score 3, 39 cows with score 4, and 7 cows with score 5. Of the 473 non-lame cows, 224 were evaluated with score 1 and 249 with score 2. Of the 356 cows used for the statistical analysis, 96 cows (27%) were categorized as lame and 260 cows (73%) as non-lame.

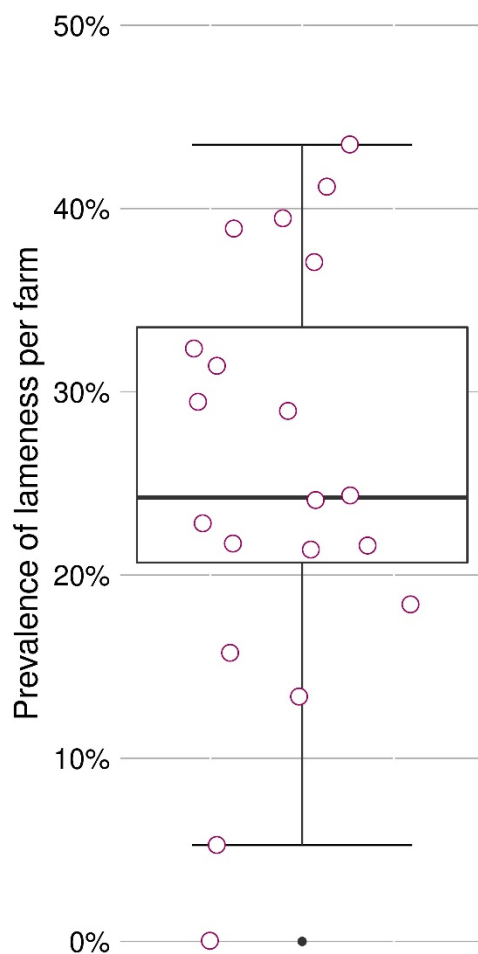


Figure 1. Prevalence of lameness across all 20 farms. Open dots show the individual prevalence per farm. The data basis for the graph was 647 cows in total, of which 174 were scored as lame cows. Lameness includes cows with locomotion scores 3–5.

Looking at the lameness prevalence per breed, 29.7% of the Holstein and 20.8% of the Brown Swiss were scored as lame.

3.2. Influence of Parity on Lameness

A positive relation between lameness prevalence and parity could be found ($p < 0.001$). With increasing parity, the percentage of lame cows increased (Figure 2). The estimated lameness prevalence within the primiparous cows was 12.6% (CI95: 6.6–22.9%). In the second parity, 19.3% (CI95: 11.1–31.5%), in the third, 21.6% (CI95: 12.4–35.2%), and in the fourth parity, 32% (CI95: 17.8–50.6%) of the cows were lame. The upward trend of the increase in lameness continued, with 51.6% (CI95: 36.2–66.8%) of the cows in the fifth and higher parity being lame.

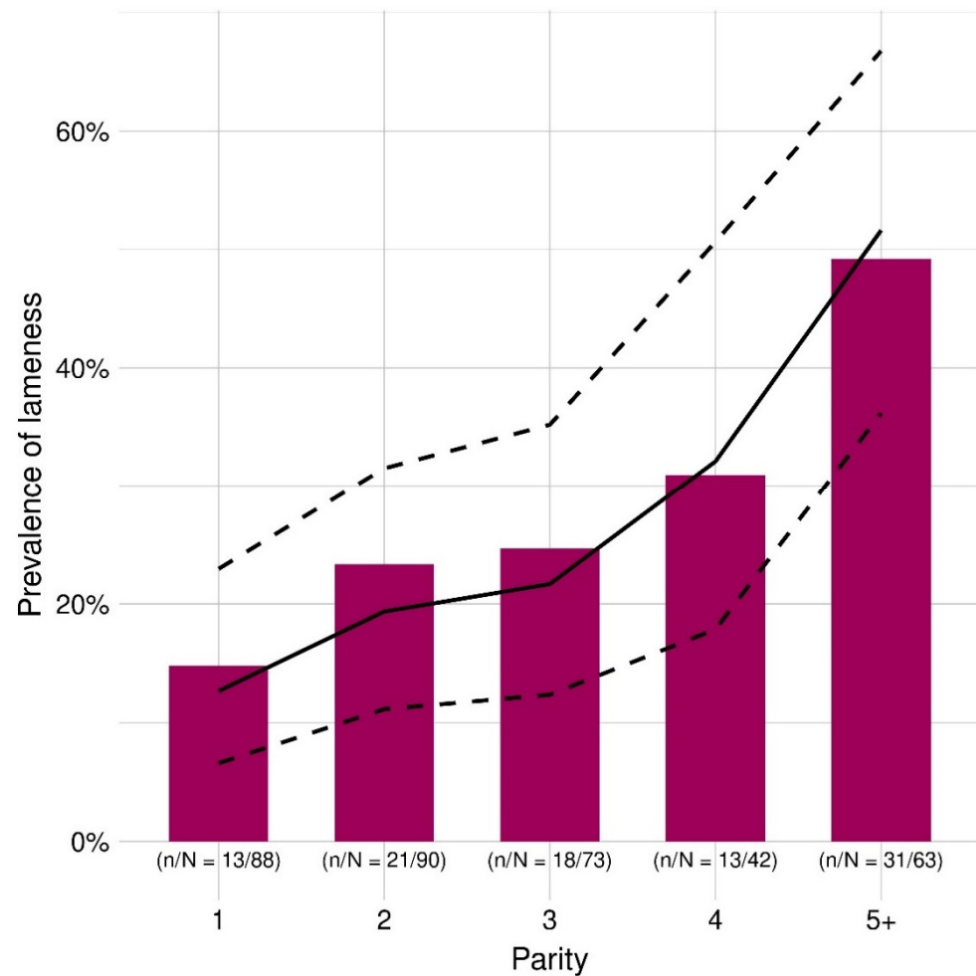


Figure 2. Prevalence of lame cows in relation to parity. Solid lines show the estimated means, dashed lines the estimated lower and upper 95% confidence interval. Graph includes the lameness status of 356 cows in total. n = number of lame cows per parity, N = total number of cows per parity.

3.3. Hind Leg Activity and Front Steps

The frequency of HLA and FS during milking was higher in lame cows than in non-lame cows (Table 3, Figures 3 and 4). The difference between the two groups was more pronounced in FS than in HLA. With increasing parity, measured HLA increased (Figure 3). In contrast, FS decreased with increasing parity (Figure 4). Regarding HLA, there was a high individual difference between the cows independent of lameness status and parity (Figure 3). This can be explained by the high variability from farm to farm of HLA (23.9%; Table 3).

Table 3. Overview of analyzed behavioral outcome variables with p -value, estimated mean \pm standard error (SE), and farm-to-farm variability as random effect.

| Behavior (per Minute) | Lameness | | | Parity | | | | | | Farm-to-Farm Variability (%) | |
|---------------------------|-------------------|-------------------|------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------|------------------------------|------------|
| | Mean \pm SE | | p -Value | 1 | 2 | Mean \pm SE | | 4 | 5+ | | p -Value |
| | Non-Lame | Lame | | | | 3 | | | | | |
| Hind leg activity | 0.844 \pm 0.064 | 0.959 \pm 0.073 | 0.026 | 0.762 \pm 0.075 | 0.869 \pm 0.075 | 0.924 \pm 0.077 | 1.019 \pm 0.086 | 0.932 \pm 0.077 | 0.008 | 23.9 | |
| Front steps | 0.810 \pm 0.048 | 1.045 \pm 0.061 | 0.00005 | 1.003 \pm 0.065 | 0.919 \pm 0.064 | 1.062 \pm 0.067 | 0.774 \pm 0.080 | 0.879 \pm 0.068 | 0.009 | 8.7 | |
| Horizontal head movements | 1.429 \pm 0.061 | 1.465 \pm 0.072 | 0.551 | 1.636 \pm 0.076 | 1.498 \pm 0.075 | 1.449 \pm 0.078 | 1.282 \pm 0.089 | 1.372 \pm 0.079 | 0.0006 | 13.4 | |
| Vertical head movements | 1.054 \pm 0.046 | 1.086 \pm 0.061 | 0.600 | 1.197 \pm 0.065 | 1.153 \pm 0.064 | 1.127 \pm 0.068 | 0.879 \pm 0.083 | 0.995 \pm 0.069 | 0.004 | 6.2 | |

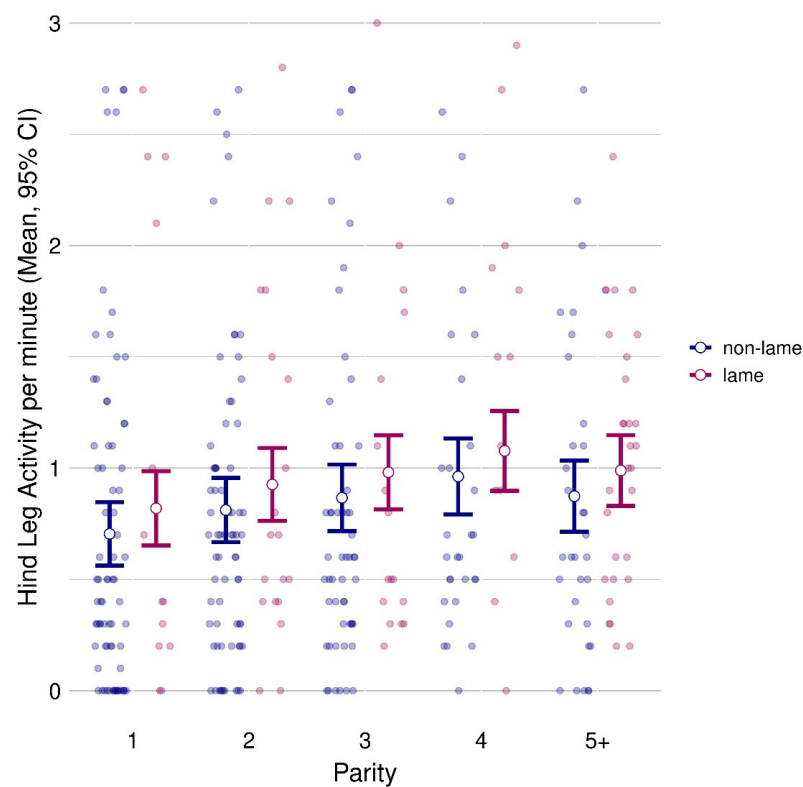


Figure 3. HLA per minute between non-lame and lame cows depending on parity. Open dots show the estimated means, error bars the estimated lower and upper 95% confidence interval, and closed dots the measured HLA of each cow.

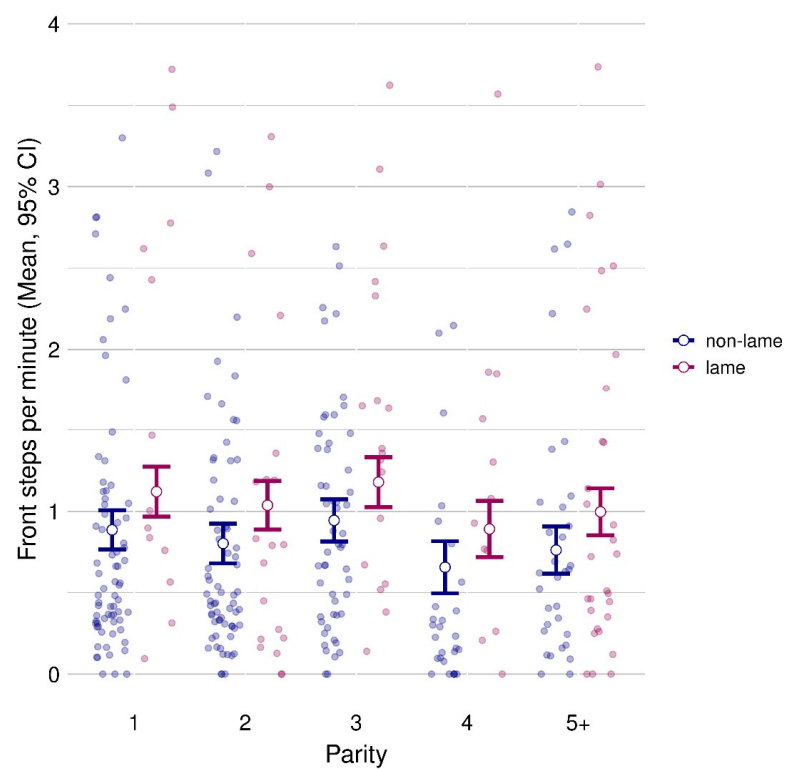


Figure 4. FS per minute between non-lame and lame cows depending on parity. Open dots show the estimated means, error bars the estimated lower and upper 95% confidence interval, and closed dots the measured FS of each cow.

3.4. Head Movements

The lameness category had no effect on HHM and VHM, but the parity did (Table 3). The frequency of HHM and VHM was less observed with increasing parity, especially from the fourth lactation onwards, analogous to the finding for FS. The farm-to-farm variability was higher for HHM (13.4%) than for VHM (6.2%; Table 3).

4. Discussion

Lameness is a very important factor in the health of a dairy herd. In Europe and North America, many studies explored the prevalence of lameness within their country in different free-stall housing systems for dairy cows. They reported, for example, a lameness prevalence of 23% in Finland [2] (5-point locomotion score with clinical lameness ≥ 3), 28.2% in the UK [42] (4-point mobility scoring with 2 and 3 clinical lameness), and 28.3% in Ontario [3] (5-point locomotion score with clinical lameness ≥ 3). With a prevalence of 27% on cow level and 95% on farm level using the same threshold for defining clinical lameness, our results fall within these ranges. In contrast, Becker et al. [1] reported a lower lameness prevalence of 15% on cow level and 81% on farm level in Switzerland. On all farms assessed by Becker et al. [1], routine claw trimming was integrated into the herd management and was performed by trained claw trimmers. Because professional routine claw trimming reduces the occurrence of lameness [43], this could be a factor for the lower lameness prevalence in their study. In our study, the participating farms were not selected by type of claw care practice, resulting in a broader range of practices being encountered. On some farms only the claws of cows that showed obvious abnormalities in the walking pattern were trimmed when recognized by the farmers. Other farmers trimmed the claws of all cows at least once or twice a year either by themselves or with the aid of claw-trimmers and additionally treated lame cows in between.

Owing to the selection criteria for our framework study, the participating farms had issues with increased somatic cell counts due to the occurrence of subclinical mastitis within their herd. This in turn may have had a negative impact on our results, as a recently published study reported that a higher herd average somatic cell count was associated with a higher clinical lameness prevalence [3]. To our knowledge, this was the first study that contradicts the findings of former publications that either could not find an association between lameness prevalence and somatic cell count [44] or found a negative correlation dependent on the herd [45]. On the other hand, there is evidence that the occurrence and control of lameness highly depends on management factors and the farmers' self-assessment [46]. Therefore, we must consider that some of the participating farms struggling with higher somatic cell count may also have a higher lameness prevalence because of management issues.

The proportion of lame cows within a herd varied greatly between the participating farms. This variability could, besides differences in the management, depend on the breed. The predominant breeds were Brown Swiss, with 20.8% being scored as lame, and Holstein, with a lameness prevalence of 29.7%. This had been found previously, reporting that Holstein cows tend to be more susceptible to lameness [3,47] or claw diseases such as Digital Dermatitis [48] than other breeds used for milk production.

Across all herds in our study, the prevalence of lameness increased with increasing parity. These results agree with the findings of Solano et al. [49], who showed an increase in lameness with increasing parity in Canadian dairy cows. Besides reproductive failure, calving difficulties, and udder health problems, lameness is one of the main culling reasons [50]. Looking at cows with more than three parities, we found a lameness prevalence above 30%. This could contribute to the fact that cows on Swiss dairy farms are only used for an average of 3.4 lactations before culling [51]. Most lameness cases are caused by claw lesions [52,53], which is the reason why the prevalence of claw lesions is used nearly synonymous to prevalence of lameness. With advancing age, the robustness of the claw horn decreases owing to postural influences. Feeding, type of flooring, housing hygiene, frequency and quality of claw care, and other diseases of the individual animal

represent only a small selection of factors that influence the development of claw lesions over time and increase the risk for lameness with increasing parity [2,54–56]. The earlier a claw problem is detected, the higher is the chance of successful cure of lameness, thereby extending the animal's productive life and improving its performance and well-being.

The challenge of early detection of claw problems is to have an approach for diagnostics that is easily integrated into the farmer's work routine and can also be supported technically. Because milking is routinely performed at least twice a day, the cow's behavior during this time, especially her stepping rate, represents a potential usable indicator and has been investigated several times. Werema et al. [17] even used weight shifting as one of four indicators in their studies to develop an alternative easy method for lameness identification during milking. As Gygax et al. [57] and Cerqueira et al. [58] found a lower stepping rate of hind legs in primiparous than in multiparous cows by visual observation, we tested our data for the impact of parity on HLA. Our results were consistent with these former findings as they show an increasing HLA in cows with increasing parity regardless of the lameness status. Trying to find an explanation for this correlation, Gygax et al. [57] suggested that older cows seem to be more prone to being unsettled by the milking situation. However, we expect that older cows are more familiar with the milking process and should therefore be calmer than younger cows. Based on our results, the increase in lameness prevalence in the cows of higher parities could be one of the reason for the increased stepping rate that Gygax et al. [57] and Cerqueira et al. [58] found.

Nonetheless, other factors influencing this finding cannot entirely be excluded. The HLA was indirectly measured by a 3D-acceleration sensor attached to the milking cluster by applying the method published by Raoult et al. [32]. These authors reported a mean HLA rate of 0.94 activities per minute during milking by direct observation and 0.86 activities per minute detected by the 3D-accelerometer. Using the same system, our estimated mean HLA rate was 0.84 activities per minute for non-lame cows and 0.95 activities per minute for lame cows. This indicates that our measurements fall into a realistic range. Because indirect measurement of HLA depends on the transmission of hind leg movements across the udder to the cluster, it could be influenced by the type of udder conformation, which changes with increasing parity [59]. Udders hang deeper between the hind legs and are less bound over the lactations [60,61], and thus swing more easily. Oscillation due to steps with the hind legs could therefore be transmitted more strongly to the milking cluster in higher parity cows compared to younger cows. Consequently, small hind leg movements of an older cow would be better detected by the sensor on the cluster than those of a younger cow. This might also result in an increase in detected HLA with increasing parity.

Further measurement inaccuracies of the method are the non-distinction between kicking and stepping, with a possible overestimation of HLA at more frequent kicking, which is not related to lameness. In addition, the number of steps cannot be counted accurately, because hind leg activities measured in an interval of less than three seconds were summed up to one activity cluster by the computer software. For example, if a cow steps five times in a row with an interval of less than three seconds in between, a direct observer will count these as five steps. The software counts the same occurrence as one activity cluster. Also, small shifting movements with the legs do not always lead to movements of the milking unit and therefore are not recognized by the sensor. This therefore leads to an underestimation of the actual HLA by the sensor measurement. A comparison to results with direct observation from other studies is consequently not reliable [32].

The intrinsic movements of the milking units with cyclic deflections of the cluster, which are also visually evident, have a significant influence on the accuracy of the measurement method. For that reason, about 40% of the collected data had to be excluded from the behavioral analysis. In these cases, the software gave too much false positive HLA.

The threshold of 0.25 g for HLA-detection was investigated in a pre-test, where sensor data from 36 milked cows were compared with the results of direct observation of the cows' movements during milking. In the main experiment, the idea was to measure HLA

with the sensors only, based on the tests of Raoult et al. [32] and the pre-test, and to focus the visual observation on the forelimb masses and the head. This is why the hind legs and the milking equipment are rarely visible in the videos. Therefore, it is not possible to conduct a sensitivity/specificity comparison between the sensor measurement and the visual observation with the data from the main experiment. For this reason, the recorded data are of limited use for improving the accuracy of the measurement technique.

As such, further investigation is needed to develop a model that can accurately distinguish movements caused by the intrinsic motion of the milking cluster from movements caused by the cow's motion. In this regard, the use of machine learning algorithms could be an option to be explored. At the same time, attaching the sensor to the milking unit has the advantage that the sensor does not have to be attached to the cow, but could be integrated into the milking system to record data on the individual cow's behavior at each milking. Even though the sensor has a low sensitivity, it showed a difference in HLA between lame and non-lame cows. Therefore, we can assume that this difference is even more pronounced with direct observation or after improving the analyzing software (additional information in this context the plotted raw data of HLA between all five degrees of lameness is presented in Appendix B).

The measured HLA differed greatly between the individual herds regardless of the lameness state. We agree with Rousing et al. [62] and Cerqueira et al. [58] that stepping and kicking behavior is highly herd dependent and has to be interpreted individually on herd level. In addition, the breed, the individual character, and the temperament of each cow may affect the behavior during standing in the parlor [63]. Therefore, only if measuring the individual cow's behavior within a herd over a longer period and using herd individual settings, as already suggested by Raoult et al. [32], could the system provide an additional assistance for lameness detection. As Werema et al. [17] only found a sensitivity of 42.1% for detecting lame cows with using weight shifting as a single indicator, it is obvious that measuring HLA could only provide assistance in detecting lameness when using other indicators at the same time. It would also be necessary to determine whether it is possible to infer lameness from behavior, but this would require establishing a threshold for the number of leg movements a cow exhibits during milking for each individual animal. On the other hand, the behavior of the individual cow would have to be analyzed over a longer period of time to determine the milking-to-milking variability. The fact that HLA of each cow was only measured during one evening milking limits the interpretation of the results regarding these aspects. Because the present study was conducted during the winter months, future studies should investigate whether movements of the cows caused by disturbing flies during the summer months significantly influence the measured values and if they can be distinguished from movements caused by weight shifting and stepping due to lameness.

Although the hind claws are more often affected by disease [53,64], the front claws can also become diseased and painful, leading to unloading by leg movements. For direct observation of the front legs, stepping and weight shifting were distinguished from kicking, because lameness does not induce kicking behavior [62], with kicking representing a defensive reaction against a sudden event. Lame cows showed a higher rate of FS during their whole stay in the milking parlor in comparison with non-lame cows, regardless of the parity. These results support the findings of former studies that showed that increased stepping and weight shifting during calm standing can be observed in lame cows [14–17]. Only Rousing et al. [62], observing all four legs, could not find a relationship between stepping during milking and lameness. Rousing et al. [62] collected data throughout the year, so it could be possible that stepping behavior due to insects in the milking parlor represented a high nuisance factor. In addition, these authors did not count the actual number of steps, but only distinguished between no, one, or more than one steps during milking. Therefore, no clear statement can be made whether the amount of stepping in lame cows differed from that in non-lame cows. It is also unclear whether weight shifting was counted as stepping as it was in our study. The number and location of affected claws could

have an influence on the cow's stepping and weight shifting behavior owing to different degrees of pain [65]. Within our data collection, it was not assessed which claw and how many claws were affected. This limits the interpretation of the results to some extent.

We hypothesized that painful claws could lead to restless behavior, which could also be reflected in an increase in head movements; this had not been previously studied. However, our results could not confirm this hypothesis. The frequency of HHM and VHM decreased with increasing parity regardless of lameness. It could be assumed that younger cows (fewer parities) are more likely to be curious about events happening in their environment than older cows (higher parities) and therefore move their head around more often to see what is happening. The observed cows were milked in auto-tandem parlors, where every cow has her own demarcated milking stall with a metal plate in front of her, limiting her sight radius. At the same time, this system allows the cows to move their head in basically every direction (up, down, backwards, right, and left). This enables the cow to see what happens in the milking pit or in the walkway next to the milking stall. When a cow moves her head up or down very often, it may also be a sign of restlessness or nervousness. The older the cows are, the calmer they may get during milking, being more used to staying patient and waiting for a longer period. This is in contrast to the assumption of Gygas et al. [57] that increasing HLA in cows with increasing parity could be due to increased unsettling by the milking situation. If the increased HHM and VHM are an indicator of restlessness as the milking is perceived as a stressful situation by the cow, we would also expect more HHM and VHM in the early lactation stage than in the later stage, when the milking process has become a daily routine again. However, we found no statistical influence of the days in milk on the number of HHM and VHM or any other behavior. Previous studies have primarily addressed differences in behavior between primiparous and multiparous cows [66], but to our knowledge none has addressed behavioral changes during milking with continuous increasing age.

In summary, an increase in HLA and FS could be seen in lame cows in comparison with non-lame cows, probably caused by the attempt to relieve lame limbs during standing in the milking parlor. This could be measured indirectly via a 3D-acceleration sensor on the milking unit with some limitations. Because lameness detection provides an important contribution to a healthy and well producing herd, this knowledge could be used in further studies on lameness detection using the changes in stepping behavior during milking. To this end, it would be necessary to verify whether the development of lameness can be detected by observing a change in the behavior of individual cows in the milking parlor. Even though the study has certain limitations due to the fact that it was not planned autonomously, the results and the indications of the limitations of the sensor provide a valuable contribution for further studies. Significant research is being conducted in lameness detection; therefore, it is important to publish methods that are not yet fully developed so that other projects can improve and learn from them.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Abbreviations

3D: three-dimensional; CI95: 95% confidence interval(s); HHM: horizontal head movement(s); HLA: hind leg activity; FS: front step(s); VHM: vertical head movement(s).

Appendix A

Table A1. Results of the preliminary test for setting a threshold for measuring HLA. TN = True negative (no HLA observed and no HLA measured), FN = False negative (HLA observed, but no HLA measured), FP = False positive (no HLA observed, but HLA measured), TP = True positive (HLA observed and HLA measured).

| Threshold [g] | TN | FN | FP | TP | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value |
|---------------|------|-----|-----|-----|-------------|-------------|---------------------------|---------------------------|
| 0.15 | 3825 | 18 | 432 | 246 | 0.93 | 0.90 | 0.36 | 1.00 |
| 0.2 | 4051 | 48 | 242 | 210 | 0.81 | 0.94 | 0.46 | 0.99 |
| 0.25 | 4197 | 76 | 125 | 181 | 0.70 | 0.97 | 0.59 | 0.98 |
| 0.3 | 4289 | 97 | 58 | 156 | 0.62 | 0.99 | 0.73 | 0.98 |
| 0.35 | 4346 | 118 | 27 | 130 | 0.52 | 0.99 | 0.83 | 0.97 |
| 0.4 | 4250 | 128 | 13 | 116 | 0.48 | 1.00 | 0.90 | 0.97 |

Appendix B

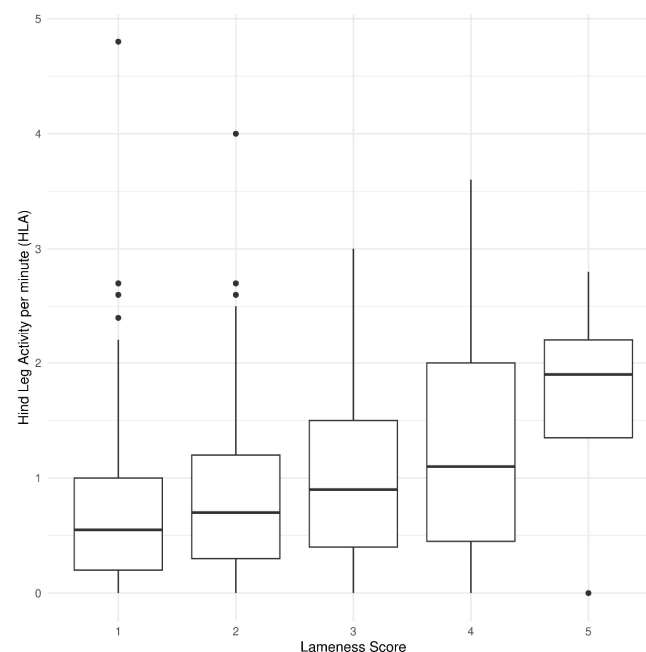


Figure A1. Raw data of HLA in relation to the degree of lameness. Measured HLA per minute at a threshold of 0.25 g plotted against assessed lameness scores of 356 cows on 17 farms. All measurements with high intrinsic motion of the milking unit were excluded beforehand.

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